

REMARKS

Claims 1 and 6 have been amended to recite that the transformed Dunaliella Salina cells are cultured in a culture fluid to provide basis for the preamble "a transgenic Dunaliella Salina bioreactor.

Support for the amendment is found in the specification at page 12, lines 1-6, page 13, lines 2-9 and page 15, line 25 to page 16, line 4.

Claim 12 has been reworded to state that the cells of Dunaliella Salina are transformed by the introduction of an expression vector containing a foreign target gene to provide transgenic Dunaliella Salina. Claims 13 and 14 have been amended respectively to delete reference to TNF cDNA fragment and to HBsAg gene fragments and claim 14 has been amended to provide antecedent basis for the recital of the fusion gene.

Support for the amendment is found in Examples 1 and 2 of the specification at pages 9 to 16. No new matter has been introduced. Entry of the amendment is requested.

The pending claims are claims 1, 6, 7, 9, 12-14.

RESPONSE

Claims 1 and 6 are amended to clearly recite a process wherein Dunaliella Salina cells are transformed by the introduction of a foreign target gene and cultured. The transformation of Dunaliella Salina provides a transgenic Dunaliella Salina useful as a bioreactor to express the foreign target gene.

Claim 12 has been reworded to state that the cells of Dunaliella Salina are transformed by the introduction of an expression vector containing a foreign target gene. Claims 13 and 14 have been amended respectively to recite that the foreign gene introduced into Dunaliella Salina are TNF cDNA and to HBsAg gene encoding amino acid residues 1-226 and HBsAg preS1 gene encoding amino acid residues 20-48 and claim 14 has been amended to provide antecedent basis for the recital of the fusion gene.

Rejection under 35 USC §112, second paragraph

Claim 12 has been rejected as lacking sense for stating "wherein the transformation is by construction of an expression vector" The claim has been amended to recite that the Dunaliella Salina cells are transformed by the introduction of an expression vector. As amended the claim clearly and specifically recites a process by which Dunaliella Salina cells are transformed to provide transgenic Dunaliella Salina to enable it to act as a bioreactor for the expression of the HBsAg. It is believed Claim 12 as amended is clear and definite and the rejection on this basis has been overcome.

Claim 14 was rejected for lacking antecedent basis for "the fusion gene" As amended, claim 14 now clearly recites a SS1 fusion gene to provide antecedent basis for "the fusion SS1 gene"

It is believed that the rejection of claims 12 and 14 on this basis has been overcome.

Rejection under 35 USC §112, first paragraph

1. Written Description

Claims 6, 7, 9 and 12-14 were rejected for failing to comply with the written description requirement.

The Examiner contends that neither a "fragment of TNF gene" nor a "TNF cDNA fragment" of claim 12 is sufficiently described in the specification. The Examiner also contends that claim 14 drawn to "amplifying a gene fragment" is adequately described in the specification.

No basis was stated for the rejection of claims 6, 7, 9 and 13. It is believed that the same contention applies to these claims.

Claim 6 as amended recites the use of the tumor necrosis factor gene or a fusion gene from the HBV surface antigen to construct an expression vector to be introduced into Dunaliella Salina cells. The introduction of the foreign target gene

into the cells of *Dunaliella Salina* provide transgenic *Dunaliella Salina* capable of expressing TNF or HBV surface antigen when cultured. There is no recital of fragment of TNF gene in claim 6. The specification provides ample description of how to construct an expression vector with the TNF gene in Example 1. The expression vector comprises a promoter, cloned chloroplast *atpA* 5', and a terminator sequence, *rbcL* 3. The TNF gene, which is about 600 bp in length and has a *BamH I* site at end and a *Xba I* site at the other end is inserted between the promoter and the terminator sequence.

Example 1 further clearly and amply describe the process of transforming *Dunaliella Salina* cells with the expression vector to provide transgenic *Dunaliella Salina* at step III on pages 11 to 12, a process of culturing the transformed/transgenic *Dunaliella Salina* to express the .

The gene of human TNF alpha is well known. Shirai T et al. described TNF as a gene of about 3.6 kb in length with 4 exons and 3 introns. The gene sequence is deposited in GenBank , X02910. The THREE domains of TNF alpha have also been clearly described by Kulski et al. as 1-34 AA for the cytoplasmic region, 35-57 AA as the transmembrane region and 59-233 as the extracellular region. The mature chain is 157 AA from AA77 to AA233 leaving a leader sequence of 76 amino acid residues. The mature TNF gene encoding the transmembrane and extracellular regions and is about 600 bp long. See Wang J. et al, J. South China Uni. Technol., 1998, 26(4):82-85; Wang A.M. et al., Science, 1995, 228(4696):149-54 and Wang A.M. et al, GenBank, M10988, 1995. A list of the publications of the cDNA and amino acid sequences of TNF is enclosed. If the Examiner requires a copy of said publications, Applicant will furnish the publications upon request.

On page 10 of the specification, lines 9-18 states clearly that the foreign target gene inserted is a 600 bp TNF cDNA with a *BamH I* site at one end and a *Xba I* site at the other end. The specification states that it is a cDNA fragment of TNF.

Example 1 provides clear and ample description for the construction of the expression vector p64C-TNF-AAAd of TNF. The cDNA of TNF that is used is clearly described as the 600 bp TNF gene that is well known to those of skill in the art as the mature TNF gene. See pages 10-11 of the specification for the details for the construction of the expression vector which is introduced to provide transgenic *Dunaliella Salina* for the expression of the target gene, TNF cDNA. Under the law, information that is well known as evidenced by the publications provided herewith need not be repeated in a specification. Thus, the specification provided ample written description of the TNF cDNA that is inserted into the expression vector.

Example 2 provides detailed description for the construction of the expression of vector containing the HBsAg SS1 fusion gene. Each step of the process is clearly and amply described. Each HBsAg gene fragment is clearly identified as that the S gene of HBV encoding 1-226 amino acid residues of HBsAg and the PreS1 gene encoding 20-48 amino acid residues of the PreS1Ag. Each step for the construction of the expression vector, the transformation of the *Dunaliella Salina* with the expression vector containing the foreign target HBsAg SS1 fusion gene, the culturing of the transgenic *Dunaliella Salina* from the transformation and the screening of the culture for the expression of the markers are clearly described. The gene sequences encoding the HBsAg has been known to those of skill in the art for at least the last forty years. Under the law, there is no requirement for providing such well known sequences in the specification to support the claimed invention.

With the full and ample description of each step of the process and with clear identification of the target genes that are to be inserted in the expression vectors, the written description requirement is met.

It may have been inartful to refer to the TNF mature gene as a cDNA fragment or that the S gene and PreS1Ag gene as gene fragments. However, the description in the specification clearly instructs the skilled in the art of the specific genes that are to be used. The written description requirement has been met by

the specific and detailed description and should be sufficient. It is believed that the rejection on this basis has been overcome by amended claim 6 and claims 7, 9, 12-14 dependent thereon.

The Examiner cites *University of California v Eli Lilly and Co.* for support of the rejection of claims 6, 7, 9 and 12-14. However, the claims of the patent in the cited case directly claim a gene sequence. Claims 6, 7, 9 and 12-14 of the present case are method claims wherein well known gene sequences are employed for the construction of an expression vector to transform *Dunaliella Salina* with the target gene for the expression of the target gene. Such method claims are distinguishable from the claim to the gene of the human insulin gene, which was not known or published at the time. Therefore the holding of the cited case is inappropriate for the rejection of the method claims at issue. In fact, the holding of the cited case has been criticized by the bar and by Judge Rader of the Court of Appeals of the Federal Circuit.

Applicant requests the withdrawal of the rejection on this basis for the reasons stated hereinabove.

2. Enablement Requirement

Claims 1, 6, 7, 9 and 12-14 were rejected for non enablement.

The Examiner criticized the wording of the claims as not having enabled a transgenic *Dunaliella Salina* bioreactor.

Applicant wish to point out that the transformation of *Dunaliella Salina* with a foreign target gene and the marker genes provides transgenic *Dunaliella Salina* when it is shown that the culturing of the transformed *Dunaliella Salina*, also referred to as transgenic *Dunaliella Salina*, and the screening of thereof demonstrated the production of the marker gene products. This is accepted by those of skill in the art as proof of the successful transformation process described. Applicant has reworded the claims to include the culturing of the

transgenic Dunaliella Salina to enable the recital of the transgenic Dunaliella Salina bioreactor.

The detailed description provided in the specification, especially that of the detailed experimental steps in Examples 1 and 2 clearly enables a person of ordinary skill in the art to practice the claimed invention. As amended, it is believed that claims 1, 6, 7, 9 and 12-14 are enabled.

It is believed that as amended the claims as presently presented are allowable and an early allowance is requested.

Applicant's attorney attempted to discuss the issues raised with the Examiner. However, no resolution was achieved. The courtesy of the Examiner is appreciated.

Respectfully submitted,
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